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Electrical properties of breast cancer cells from impedance measurement of cell suspensions

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Abstract. Impedance spectroscopy of biological cells has been used to monitor cell status, e.g. cell proliferation, viability, etc. It is also a fundamental method for the study of the electrical properties of cells which has been utilised for cell identification in investigations of cell behaviour in the presence of an applied electric field, e.g. electroporation. There are two standard methods for impedance measurement on cells. The use of microelectrodes for single cell impedance measurement is one method to realise the measurement, but the variations between individual cells introduce significant measurement errors. Another method to measure electrical properties is by the measurement of cell suspensions, i.e. a group of cells within a culture medium or buffer. This paper presents an investigation of the impedance of normal and cancerous breast cells in suspension using the Maxwell-Wagner mixture theory to analyse the results and extract the electrical parameters of a single cell. The results show that normal and different stages of cancer breast cells can be distinguished by the conductivity presented by each cell.

1. Introduction

One of the main ways to understand cell function is by the investigation of cell electrical behavior when subjected to an electric field [1] [2]. The cell membrane causes major dispersion in wide frequency bandwidth, while the dispersion is a function of the membrane permittivity. Typically, in the radiofrequency range between 1 kHz and 10 MHz many biological tissue or cells will present significant Beta dispersion [3], such multi-frequency impedance measurement is also known as impedance spectroscopy. The whole Beta dispersion range has been used to characterize cell status, e.g. cell proliferation, viability [4].

The impedance of breast cells has been studied based upon single cell measurements [5]. However, both the large cell size and shapes variation together with single cell measurement handling difficulty would introduce significant errors into the results. These shortcomings present major difficulties when the electric signatures are utilized to distinguish between normal and cancerous cells. In order to overcome these problems cell suspensions are used instead of a single cell. Measurements were taken for cell suspensions for four breast cell lines, namely: MCF-10A, MCF-7, MDA-MB-231 and MDA-MB-435S, which are: normal cell, early stage, invasive and late stage cancer cells, respectively. A special chamber designed with four electrodes connected to an HP impedance analyzer 4194A was

used for the impedance measurement of the cell suspensions. After a calibration procedure the impedance results were analyzed according to the mixture theory for two- and three-phase suspension system [6]. The electrical properties of single cell, e.g. whole cell conductivity, membrane capacitance, relaxation frequency, etc. were extracted for each type of breast cell.

2. Methods

In this research the impedance of the cell suspensions was measured using the same system introduced in the reference [6] but with the measuring chamber re-designed. Potential error sources, such as temperature variations, cell viability and volume fraction were strictly controlled during the measurement as they could have major effects on measurement sensitivity and accuracy.

2.1 Cell preparation

MDA-MB-435S, MDA-MB-231 and MDA-MB-7 (CLS-Cell lines service, Germany), and human breast tissue cell line MCF-10A (American Type Culture Collection, USA) were cultured in a DMEM/F12 media supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin, 20 ng/mL epidermal growth factor, 500 ng/mL hydrocortisone, 100 ng/mL cholera toxin and 10 µg/mL bovine insulin. For routine growth, the cells were maintained in an incubator at 37°C with 5% CO₂. For routine culture and prior to experiments, the cells were removed from the plastic cell culture dishes by digestion for 5 minutes with trypsin (Invitrogen). Cell viability was determined by counting cells on a haemocytometer using Trypan Blue exclusion (Invitrogen). The volume of cells in the suspension was measured using a centrifuge tube with 0.5 µL accuracy.

2.2 Measurement chamber

The impedance measurement of cell suspensions was carried out in a specially designed chamber (figure 1) with four electrodes, two disc electrodes for current injection and two needle electrodes for voltage pickup. Some of the chamber specifications are listed in table 1. The volume of the cell suspension required for one test was about 200 µL and the cell density was in the 10⁶ scale depending upon the controlled volume fraction of cells in the suspension.

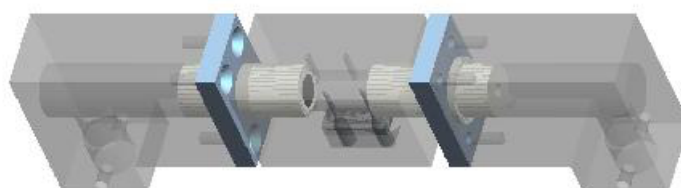


Figure 1. 3D sketch of the impedance measurement chamber for cell suspensions

Table 1. Specifications of measurement chamber

Chamber specifications			
Electrode materials	Stainless Steel	Diameter of chamber:	4mm
Disc electrode distance	11mm	Needle electrode distance	6mm tip-to-tip

2.3 Mixture theory

Theories of the two- and three-phase mixture suspension system have been studied and successfully demonstrated for analyzing cell suspension electrical properties [7] [8]. In this study the volume fraction of cells in the suspension is controlled at around 0.2, Hanai's equation (1), derived from Maxwell-Wagner equations, which are applicable for diluted suspensions, were adapted for the high volume fraction case. In order to simplify these equations, the conductivity of the cell membrane was assumed to be negligible at low frequency and infinite at high frequency, however its capacitance was treated as an important parameter and the membrane capacitance was calculated from equations 3 and 4.

$$1 - v = \left(\frac{\sigma - \sigma_p}{\sigma_2 - \sigma_p} \right) \left(\frac{\sigma_2}{\sigma} \right)^{1/3} \quad (1)$$

$$\sigma_\infty = \sigma_2 \left(1 + 3v \frac{\sigma_i - \sigma_2}{\sigma_i + 2\sigma_2} \right) \quad (2)$$

$$\tau = RC_m \left(\frac{1}{2\sigma_2} + \frac{1}{\sigma_p} \right) \quad (3)$$

$$f_0 = 1/(2 \pi \tau) \quad (4)$$

In the equations, v is the volume fraction of cells and σ is the conductivity of the cell suspension. Subscripts p , 2 and i are for the suspended cells, buffer and cytoplasm, respectively. σ_∞ is the conductivity of the cell suspension at high frequency and C_m is the membrane capacitance related to the cell radius R and relaxation time τ . f_0 is the relaxation frequency commonly used to describe β dispersion.

3. Results and Discussion

The performance of the measuring chamber was tested using the buffer with no dispersion expected in the frequency range of interest, that is: from 1kHz to 10MHz. The bandwidth of the entire measurement system was monitored and calibrated using impedance data from both buffer and known conductivity saline solutions. The cell viability (figure 2), in the measuring buffer, was tested to make sure breast cells were fully functional during the measurement at a controlled room temperature of 20°C. Rapid data acquisitions, less than one minute, were carried out for cell suspensions from the four breast cell lines.

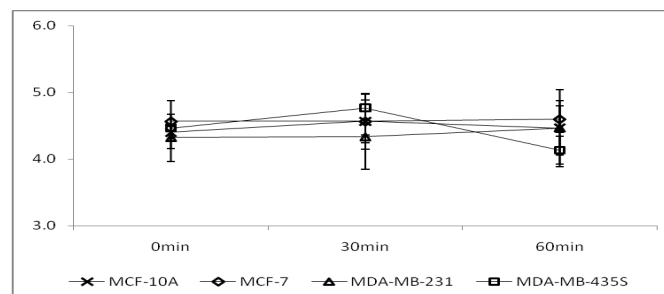


Figure 2 The relative density of four breast cell lines in modified buffer at 20°C up to 60 minutes

The results of the viability of the cell lines in modified phosphate-buffered saline (50% (v/v) D-PBS supplemented with 2% serum replacement (S2640 Serum Replacement 3, Sigma) in figure 2 showed that all the four cell lines were kept alive in the modified culture buffer up to at least one hour, at a temperature of 20°C. Electrical properties of four different single cells were then extracted from the impedance of the cell suspension and the results are presented in table 2

Table 2. Electrical properties of single cells

	MCF-10A	MCF-7	MDA-MB-231	MDA-MB-435s
Whole cell conductivity at 50kHz (mS/cm)	5.58	4.44	2.81	3.97
Cytoplasm conductivity (mS/cm)	14.04	12.99	11.68	11.84
Relaxation frequency	310kHz	600kHz	610kHz	1.01MHz
Membrane capacitance ($\mu\text{F}/\text{cm}^2$)	3.94	1.95	1.81	1.10

The results for the electrical properties of the four different breast cell lines clearly showed that each cell line had a specific electrical signature which could be utilized for identification of cancer cells and differentiation of the pathology stages of malignant cells. From the results it can be seen that the healthy cell has a higher whole cell and cytoplasm conductivity, and higher membrane capacitance than the malignant cells. On the other hand, the relaxation frequency of the four types of cell gradually increased from 300kHz up to 1MHz from normal to the late stage cancer cells, which presents progressing development natures. In the continuing research, equivalent circuits analysis and different mixture theories will be utilized to examine the results. In the future, studies will focus on the developing of this technique for cancer pathology analysis.

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